Supporting Information for:

Title: Flagella density regulates *Proteus mirabilis* swarm cell motility in viscous environments

Running Title: *P. mirabilis* cell motility in viscous environments

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Table S1. Loss-on-drying and viscosity measurements of PVP K-90 solutions.

Predicted PVP weight percent	Measured PVP weight percent	Viscosity, determined by rheometry (Pa·s)	Viscosity, determined by microparticle mean square displacement (Pa·s)
20	18.0	8.34	N.D.
10	12.1	0.83	N.D.
5	6.2	0.077	0.080 ± 0.012
2	2.3	0.009	0.011 ± 0.0023
1	N.D.	N.D.	0.0067 ± 0.0015
0.5	N.D.	N.D.	0.0038 ± 0.0007
0.25	N.D.	N.D.	0.0032 ± 0.0009
0.125	N.D.	N.D.	0.0024 ± 0.0007
0	0	0.001	0.0020 ± 0.0004

Swarm fluid	N.D.	0.061 ± 0.056

Table S2. Summary of all measurements of *flhDC*, FliC, and cell velocity.

Cell Type		WT vegetative	pACYC vegetative	pflhDC vegetative	WT elongated vegetative	WT swarm	pACYC swarm	pflhDC swarm	WT consolidated
Length (μm)*		2.5 ± 0.65	2.7 ± 0.68	3.0 ± 0.99	19 ± 5.7	19 ± 6.2	N.D.	23 ± 12	5.3 ± 2.6
flhD/*flhC* (qPCR; fold change vs WT veg)†		1.0/ 1.0	1.2 ± 0.064 1.1 ± 0.19	160 ± 5.6 79 ± 3.9	0.41 ± 0.13 0.51 ± 0.15	6.4 ± 0.043 6.2 ± 0.48	2.6 ± 1.5 2.4 ± 1.4	75 ± 3.7 51 ± 0.88	11 ± 3.7 9.0 ± 2.6
FliC (Western; fold change vs WT veg)†		1.0	1.1 ± 0.092	18 ± 2.7	1.2 ± 0.29	46 ± 7.2	48 ± 13	53 ± 3.5	14 ± 2.5
Flagella density (immuno-fluorescence; fold change vs WT veg)†		1.0 ± 0.11	1.0 ± 0.11	3.2 ± 0.28	1.1 ± 0.11	4.8 ± 0.22	5.3 ± 0.25	5.4 ± 0.32	2.8 ± 0.14
Mean velocity (μm/s)*	0.001 Pa·s	19 ± 3.5	N.D.‡	22 ± 2.6	16 ± 3.2	13 ± 2.1	N.D.	14 ± 2.4	18 ±
	0.009 Pa·s	28 ± 6.4	26 ± 6.5	34 ± 4.4	30 ± 6.3	40 ± 3.4	N.D.	35 ± 2.8	28 ±
	0.077 Pa·s	15 ± 3.8	N.D.	18 ± 3.1	14 ± 3.4	29 ± 3.5	N.D.	23 ± 2.4	14 ±
	0.830 Pa·s	4.3 ± 1.5	N.D.	6.9 ± 1.6	4.1 ± 1.1	9.9 ± 2.2	N.D.	8.6 ± 1.5	4.8 ±
	8.340 Pa·s	N.M.§	N.D.	2.0 ± 0.37	N.M.	2.4 ± 0.46	N.D.	2.5 ± 0.52	N.M.

* Data shown as mean ± standard deviation

† Data shown as mean ± standard error

‡ N.D. = Not determined

§ N.M. = Not motile

Table S3. R-squared values for correlation between cell velocity and cell length under different conditions (see Figure 3).

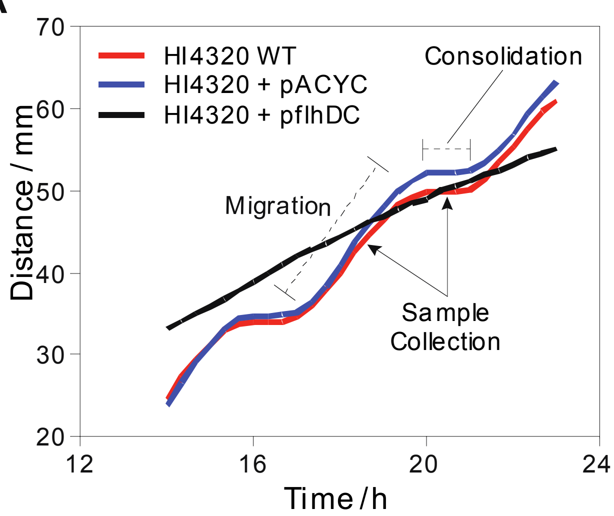
Cell Type/ Velocity	WT swarm	pflhDC swarm	WT vegetative	pflhDC vegetative	WT consolidated	WT elongated vegetative
0.001 Pa·s	0.0048	0.35	0.00039	0.017	0.00015	0.015
0.009 Pa·s	0.055	0.023	0.34	0.098	0.085	0.032
0.077 Pa·s	0.039	0.25	0.0043	0.094	0.028	0.049
0.83 Pa·s	0.10	0.023	0.0067	0.26	0.081	0.087
8.34 Pa·s	0.0012	0.17	N.A.	0.015	N.A.	N.A.

Table S4. Number and arrangement of flagella for a variety of swarming bacteria.

organism	#of flagella as a vegetative cell	fold increase in flagellation as a swarm cell
Aeromonas sp.	1 (Pol.)† (1)	many (Per.)† (1)
Azospirillum brasilense/lipoferum/irakense	1 (Pol.) (2)	many (Per.) (2)
Bacillus subtilis	several (Per.) (3)	10x (Per.) (3)
Clostridium septicum	several (Per.) (4)	many (Per.) (4)
Escherichia coli	4-7 (Per.) (5)	2-3x (Per.) (6)
Proteus mirabilis	6-10 (Per.) (7)	10-50x (Per.) (8)
Pseudomonas aeruginosa	1 (Pol.) (9)	≥ 2x (Pol.) (10)
Rhizobium leguminosarum	4-7 (Per.) (11)	3-5x (Per.) (11)
Rhodospirillum centenum	1 (Pol.) (12)	many (Per.) (12)
Salmonella enterica	6-10 (Per.) (13)	2-3x (Per.) (6)
Serratia marcescens/liquefaciens	1-2 (Per.) (14)	2-3x (Per.) (15, 16)
Sinorhizobium meliloti	2-8 (Per.) (17)	3-10x (Per.)‡ (18)
Vibrio parahaemolyticus	1 (Pol.) (19)	10-50x (Per.) (19, 20)
Yersinia enterocolitica	8-15 (Per.) (21)	1x (Per.) (21)

† Pol. = polar flagella arrangement; Per. = peritrichous flagella arrangement.
‡ WT strains of *S. meliloti* have not been shown to swarm; however, a Tn5 mutation in the *fadD* gene promotes swarming and the expression of a high density of flagella.

A



B

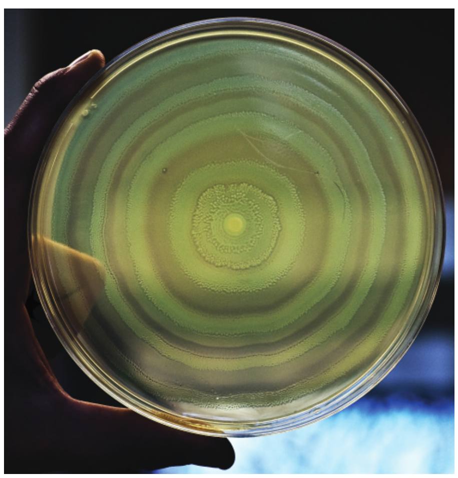


Figure S1. A plot depicting the growth of a swarming colony of *P. mirabilis* on 1.5% (w/v) agar. (A) The swarm colony is actively migrating during a swarming phase and is stationary during a consolidation phase. However, overexpression of *flhDC* (HI4320 pflhDC) produces a swarm colony that lacks consolidation phases. We collected cells for motility studies at the indicated times during swarming and consolidation phases. Each point represents the average of three independent experiments. (B) The periodic cycling between swarm and consolidated cells is hypothesized to produce the characteristic terraced pattern of a swarming colony of *P. mirabilis*.

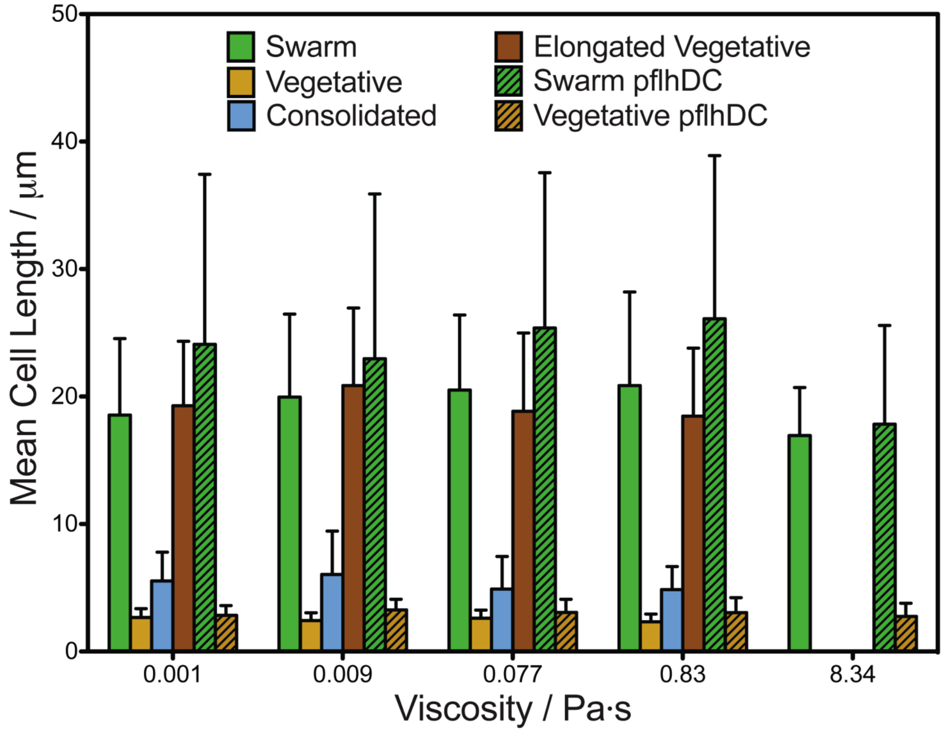


Figure S2. A plot depicting the mean cell length for each cell morphology in a range of viscous solutions. This graph shows that within a given cell population, the length distribution of the cells used for velocity analysis was consistent in each of the viscous motility buffers. Bars represent the mean of 100 individual cells. Error bars represent standard deviation of the mean.

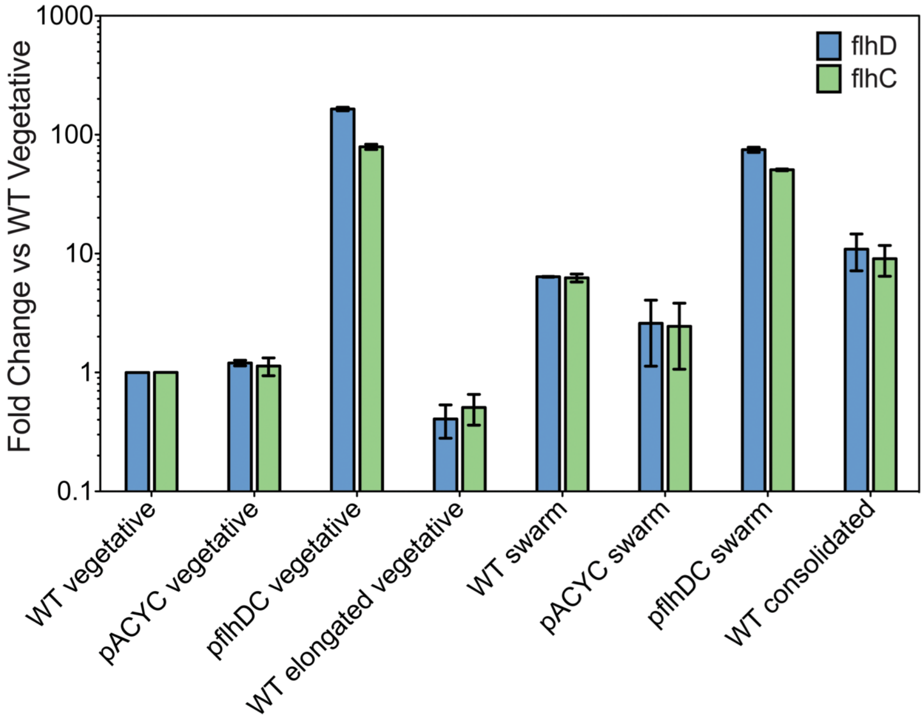


Figure S3. A plot depicting quantitative PCR that demonstrates both swarm and consolidated cells express more *flhD* and *flhC* than vegetative cells. Cells containing pflhDC have higher *flhD* and *flhC* mRNA levels. All data is normalized to the total mass of RNA. Bars represent the mean of 2 independent biological replicates. Error bars represent the standard error of the mean.

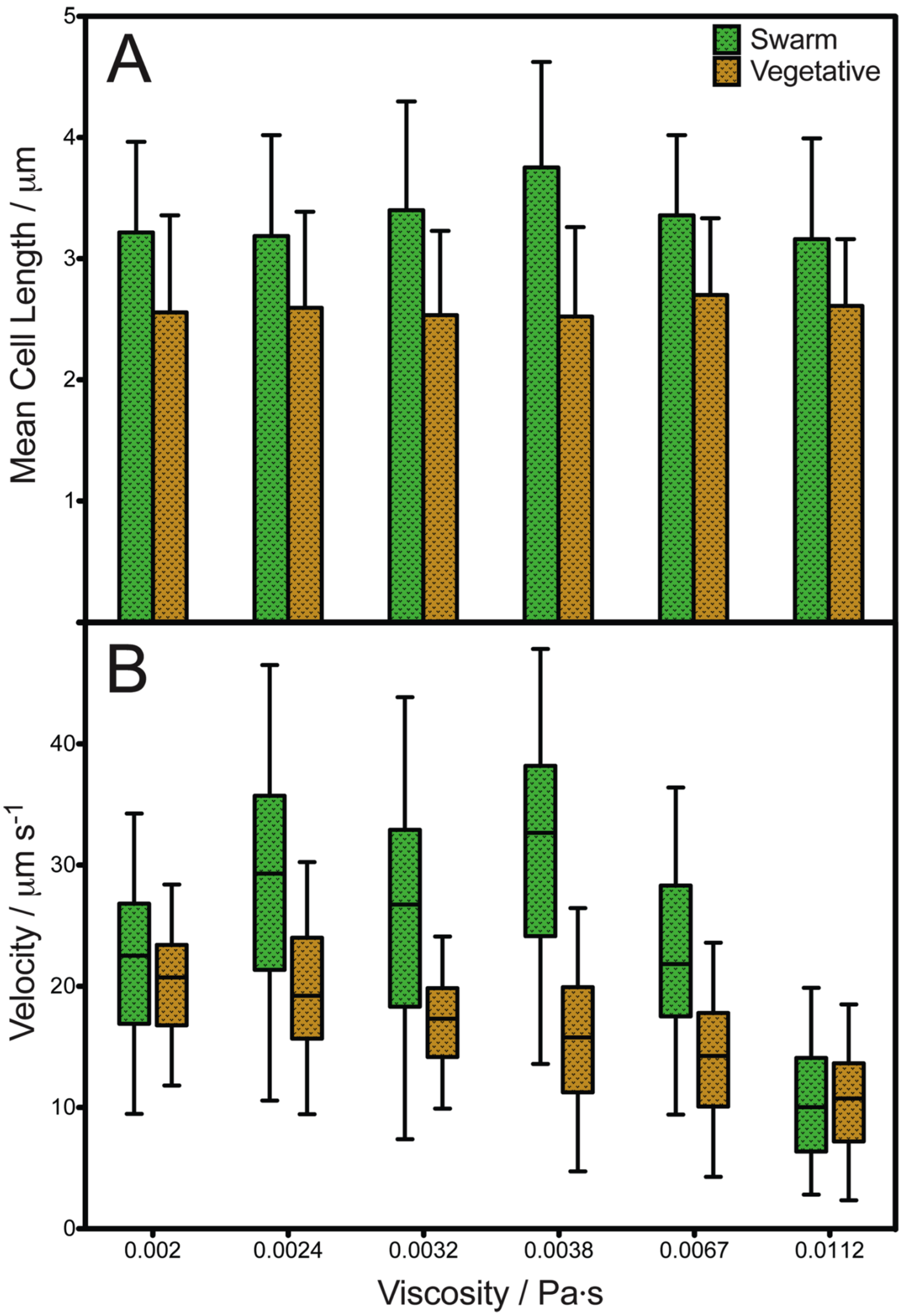


Figure S4. (A) Mean cell length for swarm and vegetative *E. coli* cells in a range of viscous solutions. This graph shows that within a given cell population the length distribution of the cells used for velocity analysis was consistent in each of the viscous motility buffers. Error bars represent standard deviation of the mean. (B) Swimming velocities of *E. coli* swarm and vegetative morphologies in motility buffers with a range of viscosities. These data are displayed as box-and-whisker plots with a box representing the range from the 25^{th} to the 75^{th} percentile and a horizontal line within the box representing the median. The error bars denote 1.5 times the interquartile distance. Each point represents measurements of between 46 and 258 individual cells. Note: In all other figures, the plotted viscosity values are from rheological measurements of the fluids, while this figure uses the values determined by bead diffusion. Therefore, 0.002 and 0.011 Pa·s in this figure correspond to 0.001 and 0.009 Pa·s, respectively, used in other figures.

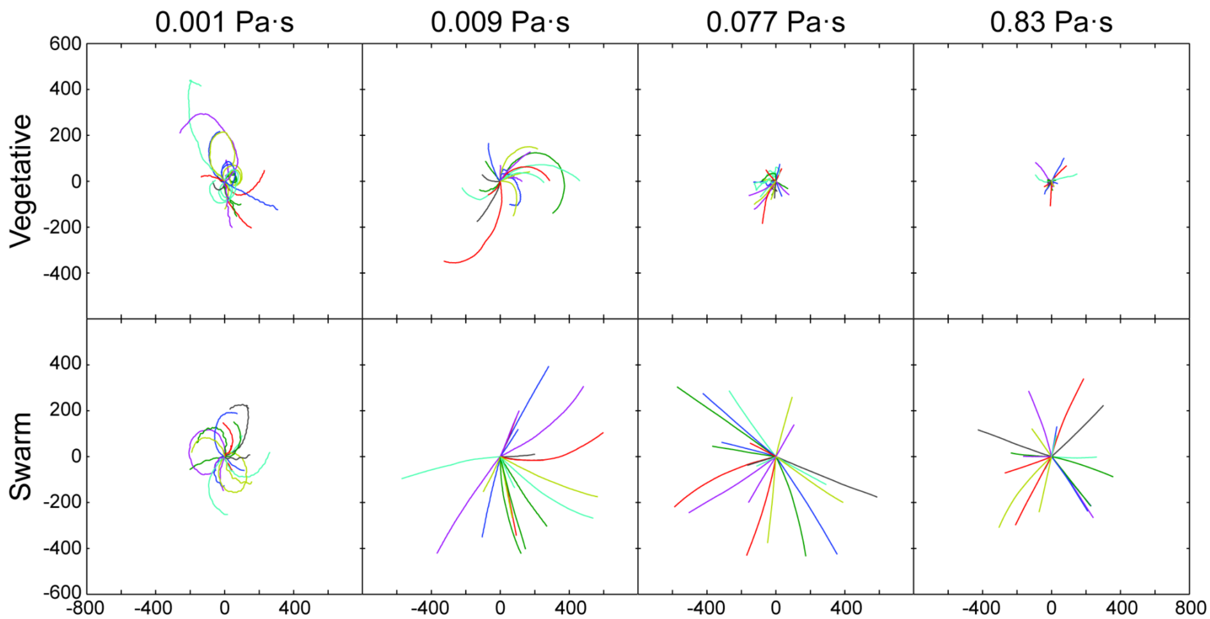


Figure S5. Representative trajectories of vegetative and swarm cells in various motility buffers. Each plot comprises 20 trajectories plotted such that the beginning of the track occurs at coordinates (0,0). Swarm cell tracks are generally straighter than vegetative tracks, likely due to longer cells having less rotational diffusion.

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